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# Effect of cefoperazone on the pharmacokinetics of methotrexate in the rabbit

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**Abstract**

The effect of cefoperazone (CPZ) on the pharmacokinetics of methotrexate (MTX) was studied in 10 rabbits. MTX was administered as a bolus dose of 2 mg $kg^{-1}$, followed by a constant infusion of 50 $\mu g\ kg^{-1}\ min^{-1}$ for 120 min. A group of 5 rabbits received, concomitantly, 20 mg $kg^{-1}$ CPZ every 30 min, begining at MTX administration. Blood samples were serially collected for a total period of 320 min post infusion, and plasma was analyzed for MTX using high performance liquid chromatography (HPLC). The mean ($\pm$ SD) values of the pharmacokinetic parameters were as follows: total area under the curve (AUC) $967 \pm 704$, $1801 \pm 778$ $\mu g\ min\ l^{-1}$; steady state MTX concentration ($C_{ss}$) $6.2 \pm 4.1$, $9.9 \pm 3.6$ $\mu g\ ml^{-1}$; and maximum plasma concentration ($Cp_{max}$) $7.5 \pm 4.5$, $24.2 \pm 11.2$ $\mu g\ ml^{-1}$ for the single and drug combination groups, respectively. The differences in these parameters were statistically significant ($p < 0.02$). The corresponding estimates of elimination half-lives ($t_{1/2}$) were $60 \pm 21.6$ and $53.3 \pm 8.5$ min, and the difference in the value of this parameter between the treatment groups did not reach statistical significance ($p > 0.05$). These results suggest that CPZ may influence the distribution phase, rather than the elimination stage, of MTX disposition in rabbits. The results of the present study may have relevance to a potential interaction that can exist between these drugs when administered concurrently in humans. However, further studies in appropriate subjects are needed to verify and characterize the clinical significance of such an interaction.

*Keywords:* Methotrexate; Cefoperazone; Interaction; Pharmacokinetics; Rabbits; Infusion

## 1. Introduction

Methotrexate (MTX) is widely used in the treatment of various malignant and non-malignant diseases (Bertino, 1981). Several drugs have been reported to delay the elimination of MTX and therefore enhance its potential toxicity (Nierenberg and Mamelok, 1983). In both the rabbit and man, renal and biliary excretion of MTX accounts for more than 70% of its total body clearance (Iven et al., 1985). Cefoperazone (CPZ), a third generation cephalosporine, commonly used for the treatment of infections in cancer patients (Craig and Gerber, 1981), is a drug which may be prescribed with MTX. Like MTX, CPZ does not appear to be significantly metabolized (Craig and Gerber, 1981), although some degree of CPZ degradation in plasma has

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been reported (Najjar, 1992). Most of the drug interactions with MTX have been reported to occur through competition for renal and biliary excretions (Iven and Brasch, 1986; Iven and Brasch, 1990; Kates and Tozer, 1976). The similarity of the disposition properties of MTX and CPZ suggest a potential interaction between these agents. The present study in rabbits was designed to investigate such a possibility.

## 2. Material and methods

### 2.1. Pharmacokinetic study

Ten male New Zealand white rabbits weighing between 2.3 and 4.9 kg were assigned to two groups (A and B). Two cannulas (Terumo, 22G × 1, i.d. 0.60 × 25 mm) were placed in the marginal ear veins (one in each ear) for the administration of CPZ and/or MTX. A third cannula was placed in the central ear artery, opposite the site of MTX administration, and used for blood collection. MTX (Lederle, Cyanamid of Great Britain Ltd, UK) was prepared in 5% dextrose solution containing 0.2% sodium bicarbonate and infused with the use of a perfusion pump (Terfusion, Model STC-502) at a rate of 50 $\mu$g kg$^{-1}$ min$^{-1}$ for a period of 120 min. Prior to an infusion session with MTX, a loading dose of 2 mg kg$^{-1}$ MTX was given as a bolus injection. This initial dose of MTX was selected on the basis of the expected steady state drug level multiplied by (0.2 l kg$^{-1}$), the steady state volume of distribution of the drug. The animals in group B simultaneously received an intravenous dose of CPZ 20 mg kg$^{-1}$ every 30 min for the duration of the study. Each dose of CPZ was freshly prepared in normal saline prior to its administration.

Blood samples of 2 ml were serially collected before, and at, the following time intervals; 5, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 230, 260, 290 and 320 min from the start of MTX infusion. Plasma was subsequently separated and stored at −20°C until the day of analysis.

### 2.2. MTX analysis

A high performance liquid chromatographic (HPLC) method, developed in our laboratory, was used to measure MTX concentrations in the plasma samples (Najjar et al., 1992). The system used consisted of a 720 system controller, a 730 data module, a 710B automatic injector (Wisp), and a 481 UV detector (Waters Associates, Milford, MA, USA). Initially, plasma samples were deproteinized with trichloroacetic acid. An aliquot of 100 $\mu$l of the supernatant was then injected onto a C-18 Novapak column. The separation was completed with a mixture of phosphate buffer, methanol and acetonitrile (84:11:5), which was pumped at a flow rate of 2.3 ml min$^{-1}$. The effluent was monitored at 313 nm, where MTX and the internal standard (4-amino-acetophen) appeared at 5.3 and 8.8 min, respectively.

## 3. Data analysis

The pharmacokinetics parameters (maximum temperature, $T_{max}$; maximum plasma concentration, $Cp_{max}$; $\beta$; area under the curve for at a given time, $AUC_{0-t}$; total area under the curve, $AUC_{0-\infty}$; and MRT) were determined using the software PCNONLIN (Version 4.0, SCI, ClinTrials, Lexington, KY, USA). The data were fitted using a non-compartmental model for constant infusion input (Model 202). The total body clearance (TBC) and the volume of distribution at the steady state ($Vd_{ss}$) were calculated from the following relationships:

$$TBC = K_0/C_{ss}$$

$$Vd_{ss} = [K_0T(AUMC)/(AUC)^2] - [K_0T/2AUC]$$

The data were expressed as the mean ± SD. A paired $t$-test was performed to determine the level of significance, with a $p$ value of $< 0.05$ taken as the limit of significance.

## 4. Results

Fig. 1 shows the mean ± SD plasma MTX concentration versus time curves for the single and drug combination groups of rabbits. MTX constant infusion proceeded with an i.v. bolus of a dose equal to 2 mg $kg^{-1}$. In all rabbits, a steady state was reached within the first 20–40 min post infusion. The concentration of MTX at the 5 min time point, representing $Cp_{max}$, was about 21 and 144% above $C_{ss}$ for groups MTX and MTX + CPZ, respectively. The pharmacokinetics parameters and statistics are shown in Table 1. A significant difference ($p < 0.02$) was observed for the $Cp_{max}$, $C_{ss}$, $AUC_{0-t}$, $AUC_{0-\infty}$, MRT and TBC in the two groups of rabbits. The increase obtained in those parameters averaged from 60 to 100% above the control group. The remaining parameters, such as the elimination rate constant and the half-life, did not change significantly ($p < 0.5$).

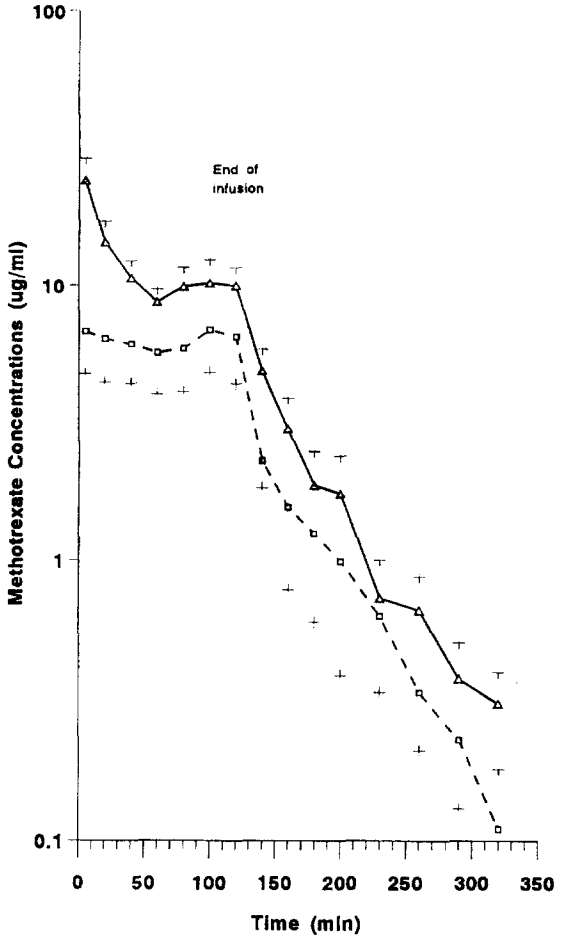


Fig. 1. Plasma concentration versus time curves for methotrexate (MTX) after an i.v. bolus of 2 mg $kg^{-1}$ MTX, followed by a constant infusion of 50 $\mu g\ kg^{-1}\ min^{-1}$ for 120 min (▲, ■). Cefoperazone (CPZ) was injected intravenously at a dose of 20 mg $kg^{-1}$ repeated every 30 min for the duration of blood sampling (▲). Each point represents the mean ± SD obtained in 5 rabbits.

Table 1
Mean (± SD) values for the pharmacokinetic parameters of MTX following its administration alone, or in combination with, CPZ ($n = 5$ rabbits)

| Parameter | MTX | MTX+CPZ | Statistics |
|---|---|---|---|
| $Cp_{max}$ ($\mu g\ ml^{-1}$) | 7.5 ± 4.5 | 24.2 ± 11.2 | <0.02 (S) |
| $C_{ss}$ ($\mu g\ ml^{-1}$) | 6.2 ± 4.1 | 9.9 ± 3.6 | <0.01 (S) |
| $K_d$ ($min^{-1}$) | 0.0129 ± 0.005 | 0.0133 ± 0.002 | >0.5 (NS) |
| $t_{1/2}$ (min) | 60 ± 21.6 | 53.3 ± 8.5 | >0.5 (NS) |
| $AUC_0$ 320min($\mu g\ min\ l^{-1}$) | 962 ± 701 | 1793 ± 771 | <0.01 (S) |
| $AUC_{0-\infty}$($\mu g\ min\ l^{-1}$) | 967 ± 704 | 1801 ± 778 | <0.01 (S) |
| MRT (min) | 28.5 ± 9.4 | 20.7 ± 7.8 | <0.01 (S) |
| TBC ($l\ min^{-1}$) | 20 ± 3.1 | 37 ± 20.4 | >0.1 (NS) |
| $Vd_{ss}$ (l) | 1.08 ± 0.19 | 2.55 ± 1.5 | >0.05(NS) |

S = significant and NS = not significant.

## 5. Discussion

The dosing regimen of CPZ (20 mg $kg^{-1}$ every 30 min) was selected based on its elimination half-life ($t_{1/2}$) in rabbits, which is about 30 min, and on the dose used by others (Hayashi et al., 1986). The average plasma concentration predicted with this dosing regimen is about 100 $\mu g\ ml^{-1}$, which is close to the average steady state concentration predicted in humans.

As shown in Fig. 1 and Table 1, the group of rabbits treated with CPZ show significant changes in the plasma concentration versus time curve profile for MTX during the infusion period. *Cp-*

$_{max}$, $C_{ss}$ and $AUC_{0-\infty}$ increased by about 144%, 60% and 86%, respectively, above the control group. The elimination rate constant ($K_d$) was determined from the data obtained after the completion of drug infusion. The results did not show a statistically significant difference ($p > 0.05$). MTX cytotoxic effects are a function of both drug concentration and duration of exposure (Pinedo and Chabner, 1977). Because the concentrations of MTX rise during infusion by more than 50%, and because infusion is a commonly used route for MTX adminstration, patients should be monitored carefully when CPZ is combined with MTX.

The exact mechanism for this interaction cannot be precisely stated. However, because there was no change in $K_d$, the interaction may not be due to competition at the level of elimination. It is possible that the changes observed were due to the effect of CPZ on the disposition of MTX. The presence of a higher $Cp_{max}$ in the animals treated with CPZ may be good evidence that the effect was on the distribution of MTX. As comparative evidence, the interaction between triazolam and cimetidine, which includes changes in the AUC but not in the $t_{1/2}$, was explained as an effect on the disposition of cimetidine (Cox et al., 1986). The two drugs (CPZ and MTX) are widely distributed in body tissues and, in particular, the distribution of MTX in the erythrocyte (Lee et al., 1986) could be affected by the concurrent administration of CPZ due to its high binding affinity (Craig and Gerber, 1981). The effect of CPZ on the pharmacokinetics of MTX is limited to the study conducted by Iven and Brasch (1990). In this study, it was reported that CPZ increased the renal clearance of MTX and its metabolite 7-OH-MTX. Such a conclusion could be rejected based on several points. The clearance of MTX was determined based on the data obtained during infusion, and this may not reflect the elimination process. Furthermore, the investigators in this study reported that the decline in the MTX steady state level was not maintained by increasing the dose of CPZ. Instead, a decrease back to the predrug values was observed. The effect of cephalosporines and piperacillin on the disposition of MTX has been reported in the literature (Iven and Brasch, 1986, 1990).

In conclusion, CPZ administered concomitantly with MTX alters the pharmacokinetic parameters of the AUC, $C_{ss}$ and $Cp_{max}$, but it had no significant effect on $K_d$. The anticipated interaction at the elimination phase did not materialize, despite the stated relevance of the renal and biliary excretion processes to the overall drug elimination processes for both agents. However, further studies in appropriate subjects are needed to verify and characterize the clinical significance of such an interaction.

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